## Forest detective searches for "animal pollen"

by Ed Berg

Animal pollen? There is no such thing: flowers—not animals—produce pollen. OK, but let's ask if animals could produce something like pollen? Is there some kind of "pollen analogue" for animals?

Here is why the forest detective considers this question interesting. Pollen is like a time-travel machine for plants. Pollen is almost indestructible and it preserves very well in sediments at the bottom of lakes. As time passes, mud and pollen accumulate on lake bottoms like pages in a diary written over thousands of years. Unfortunately, the lake mud pollen diary is only about plants. I would like a similar diary about animals.

To see the power of this methodology, at least for plants, let me explain a bit about the mechanics of how it works. To collect lake sediments we extract a cylindrical core of mud by pushing a thin-walled tube down into the lake bottom. The tube is then pulled up, and the sediments are gently pushed out of the tube as a solid core. If the sediments are mushy, especially near the top, they are collected in small plastic bags, at 1-centimeter intervals.

To read the sediment diary we first extract the pollen with various acids and sieves, and then identify and count the pollen grains under a microscope. We might do this for 20 to 50 evenly-spaced intervals in a core, depending on interest and available funding.

Next, the mud layers must be dated using radiocarbon (C-14) dating. This dates the pages of the diary, and it is absolutely necessary. Visibly, the mud is clueless about its age: it could be 600 years or 6000 years or 60,000 years old.

Once the diary is dated, we have a story of the comings and goings of plant species around the lake for thousands of years. For example, in 1998 we took a 9-meter core from Paradox Lake, twelve miles north of Sterling. Scott Anderson and co-workers from Northern Arizona University have analyzed the pollen in this core and developed a 16,000-year record of vegetation succession since the end of the last ice age. In this core we can see that white and black spruce arrived about 8500 years ago. Prior to spruce forest, the countryside was shrub tundra covered by dwarf birch and willow, with lots grass, sage, and shrubs of the

blueberry-cranberry-Labrador tea family.

Scott Anderson also made a detailed study of forest fire charcoal in this core, counting the charcoal grains in 900 one-centimeter cookies sliced from the core. He estimated that fire frequency was low in the post-glacial tundra period, increased as shrubs became more widespread, and peaked when white spruce established. The fire peak generally tracks the Hypsithermal warm period of 7-10,000 years ago, which we know from other studies was a warm and dry period, with July temperatures for southern Alaska estimated at 2 to 7°F warmer than now. With the onset of cooler climate and a modern mixed spruce-birch forest, the fire frequency has dropped to nine fires per thousand years for the last several thousand years.

So, you can see that sediment cores can tell us a lot about the vegetation and fires, because pollen and charcoal preserve so well. Unfortunately, we don't yet know how to read an animal story in these sediments. That is why we need "animal pollen."

Here are some questions that animal pollen might answer: when did moose arrive on the Kenai? Did we have many moose before European settlement? Historical records suggest that there were not many moose on the Kenai before late 19<sup>th</sup> century fires. For caribou, we know that the Dena'ina supplemented their salmon-based diet with caribou in the winter. When did the caribou arrive and did their populations rise and fall? What is the history of Kenai salmon and how did this affect the ebb and flow of different native cultures over the last 8-10,000 years that humans have inhabited the Kenai? What have the spruce bark beetles and other forests pests been doing for the 8500 years that spruce trees have been around to eat?

What might work as an animal analogue for pollen? You would think that there would be something insoluble in moose or hare pellets that would preserve through time, but alas those pellets seem to return all too efficiently to the soil from which they sprang.

Even though pellets don't preserve, however, it might be possible to find a record of fungi that live on the pellets. Fungi produce spores, which preserve almost as well as pollen. Researchers in the Rio Grande basin have recovered spores of the dung fungus Sporormiella from lake sediments. The abundance of these spores declined sharply at the end of the last ice age, right at the time of the massive die-off of large ice-age mammals, such as bison, horse and mammoths. The spores reappeared when humans brought domestic grazing animals into the area several hundred years ago. If moose are a recent addition to the Kenai wildlife, we would expect a lake core to show a rise in dung fungus spores after their arrival.

We might also consider parasites. Some insects produce hard parts that preserve well in sediments. Scott Anderson, for example, found a 10,000-year record of caterpillar head capsules from spruce budworm (or a close relative) in sediments in a pond in Maine. The head capsules which look like tiny football helmets preserve just like pollen, and are extracted and counted in the same way. It would be nice, for example, if warble fly larvae which live under caribou skin produced some preservable hard parts. Goodness knows, enough warble flies are produced when 20,000 caribou pass by a pond in the arctic!

Midges (such as "no-see-ums" and non-biting chironomids) preserve well in sediments, and indeed this summer Canadian entomologist Ian Walker will be looking for fossil midges in Kenai Lake sediment cores as indicators of past climate. It turns out that midges are good water temperature recorders, with each species having its preferred temperature zone. Midges, unfortunately, are not associated with any particular animal species, so they tell us nothing about specific animals, although they are a good indicator of potential fish habitat.

In the past I always figured that the quest for animal pollen was something like seeking the Holy Grail. But now the Grail may be in sight, at least partially. Last spring as I was driving to work one morning I heard on the radio about a study that used fossil DNA in soil to look for extinct animals. This could be better than pollen, which in plants is often hard to identify to the exact species. Fossil DNA goes straight to the genetic identity of an organism; there's nothing closer than DNA. When I heard this report on the radio, I cried, "Eureka!" and have been excited about the idea ever since.

DNA is a fairly tough molecule, compared to protein, and it sometimes survives well in fossils, especially in plant fossils, "Jurassic Park" notwithstanding. The idea that animal DNA might survive in soils, however, is a radical proposal, way out in left field. Soil sci-

entists like to think of soil as a living organism, filled with smaller organisms that are constantly ingesting, digesting, and excreting mineral and organic particles. Soil is not a favorable habitat for preserving big organic molecules like DNA and certainly not for proteins, carbohydrates, and fats. This is the biological meaning of, "Ashes to ashes, dust to dust," heard at funeral services.

The report that I heard last spring described fossil DNA in frozen permafrost soil from Siberia and in cave soil from New Zealand. The permafrost soil yielded DNA from extinct wooly mammoths, bison, and horses, as well as from lemmings, hares, reindeer, and musk oxen, dating to 30,000 years ago. Plant DNA in deeper permafrost preserved much better, and yielded 19 plant types dating up to 400,000 years ago. The New Zealand cave soil dated to 3,000 years ago, and contained DNA from 29 plant types, as well as three types of extinct flightless birds called moas, and an extinct parakeet.

This soil DNA study, by geneticists Eske Willerslev from Denmark and Alan Cooper from New Zealand is a major milestone in paleoecology. Alan Cooper has received ample funding to set up a state-of-theart DNA lab, called the Ancient Biomolecules Centre at Oxford University in England, and I am sure that we will be hearing a lot more about this kind of work in the future. As an application of DNA, it could well do for the study of past life what DNA has done for police work and criminal identification.

Unfortunately, the soil DNA method is presently limited to more-or-less protected soils, where the destructive activity of soil organisms has been inhibited, either by freezing (as in permafrost) or drying (as in cave soils). The animal DNA in soil probably comes from feces, where cells have sloughed off the gut wall and been carried out with the feces. The fecal material could easily be washed into lakes and accumulated in the mud, but its DNA might be damaged in the process.

Some lakes do not have an annual turnover of the water, which means that the bottom water is anoxic (no oxygen) and only very specialized kinds of organisms (such as anaerobic bacteria) can survive. This might be a better place to look for DNA in the sediments, than in a normal lake with lots of bottom dwellers processing the mud. Paradox Lake, where we have focused our pollen and charcoal studies, is a deep (51 feet) anoxic lake and would be a good candidate. It could potentially record 16,000 years of moose poop, if moose have been around that long. Humans, too, shed

some DNA, and there could be a DNA record of ancient lakeshore campers in that mud.

Soil DNA is about as close to "animal pollen" as the forest detective has been able to come. It's not as user-friendly as plant pollen, but it could be a great start.

Ed Berg has been the ecologist at the Kenai National Wildlife Refuge since 1993. Thanks to Scott Anderson, Ted Bailey, Tony Fischbach, Edward Mitchell and Linda Reinink-Smith for helpful comments on this article. Ed Berg will be teaching his 1-credit "Cycles of Nature" course at the Soldotna and Kachemak Bay campuses of the Kenai Peninsula College, starting March31 and April 1, respectively. Recent bird sightings are on the Central Peninsula Birding Hotline (907) 262-2300. Previous Refuge Notebook columns can be viewed on the Web at http://www.fws.gov/refuge/kenai/.